(a) introducing into host cells one or more vectors comprising a polynucleotide expressing said protein;

(b) culturing a first aliquot of the host cells with fatty acid substrate of said protein and with an agent being tested as an inhibitor of said protein;

(c) culturing a second aliquot of the host cells with fatty acid substrate of said protein;

(d) measuring, in the first and second aliquots, uptake of the fatty acid substrate of the host cells;

wherein less uptake of the fatty acid substrate in the first aliquot compared to the second aliquot is indicative that the agent is an inhibitor of said protein.

Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages iii-vi).

REMARKS

This application was originally filed on September 23, 1999 with 81 claims. An Office Action mailed from the United States Patent and Trademark Office on March 26, 2001 contained a seven-way restriction requirement. In the response dated June 26, 2001, Applicants elected the claims of Group III, Claims 19, 23, 27, 29, 31, 33, 35, 55-57, 59, 67, 69, 71, 73-74, 78 and 80-81, for prosecution in this application. Applicants also cancelled Claims 1-72, 75-77 and 79, amended Claim 73 and added Claims 82-123 in that response. The Office Action mailed from the United States Patent and Trademark Office on October 1, 2001 contained an additional requirement requiring restriction to a single invention of nucleic acids selected from the specified groups (a) - (k). In the response dated November 1, 2001, Applicants elected the nucleic acid with SEQ ID NO: 24 and requested rejoinder of the nucleic acid with SEQ ID NO: 46 and the amino acid with SEQ ID NO: 25. The request for rejoinder was granted in the Office Action mailed from the United States Patent Office on March 27, 2002.

Prior to entry of this Amendment, Claims 73, 74, 78 and 80-123 are pending. Claims 98, 99, 102, 103, 105, 106, 108, 109, 112, 113, 115, 116 and 119-122 have been withdrawn as

directed to a non-elected invention, and Claims 73, 74, 78, 80-97, 100, 101, 104, 107, 110, 111, 114, 117 and 118 are rejected.

In this Amendment, Applicants have cancelled Claims 73, 74, 78, 80 and 81, without prejudice, and have amended Claims 100, 101, 104, 110, 111 and 114. Support for the amendments to the claims can be found throughout the specification, examples, figures and claims of the application as originally filed. Specifically, support for the amendments to Claims 100, 101, 104, 110, 111 and 114 can be found at least in the originally filed claims. No new matter has been added as a result of the amendment to the claims.

The various informalities, objections and rejections contained in the Office Action are addressed in the following paragraphs in the order in which they appear in the Office Action.

Rejoinder of Claims Encompassing SEQ ID NO: 25 and SEQ ID NO: 46

Applicants note with great appreciation that their request for rejoinder of the claims encompassing SEQ ID NO: 25 and SEQ ID NO: 46 has been granted, and that these claims will be examined in addition to those encompassing SEQ ID NO: 24.

Objection to Specification for Inclusion of URL's

The disclosure was objected to because it contained browser-executable code, "URL's", for example, at page 33, line 6. The specification has been amended to delete all occurrences of browser executable code, and to replace all such references to websites with a non-executable description. Withdrawal of the objection is respectfully requested.

Objection to Application Title

The title has been objected to on the grounds that it is "not descriptive". As suggested by the Examiner, the title has been amended to "Methods Of Identifying Inhibitors of Fatty Acid Transport Proteins (FATP)". Withdrawal of the objection is respectfully requested.

Rejection of Claims 73, 74, 78, 80-97, 100, 101, 104, 107, 110, 111, 114, 117 and 118 and Claims 104 and 114-116 Under 37 C.F.R. 112, First Paragraph

Claims 73, 74, 78, 80-97, 100, 101, 104, 107, 110, 111, 114, 117 and 118 and Claims 104 and 114-116 have been rejected under 37 C.F.R. 112, first Paragraph because, according to the Examiner, "[T]he specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims".

Specifically, with regard to Claims 73, 74, 78, 80-97, 100, 101, 104, 107, 110, 111, 114, 117 and 118, at page 4 of the Office Action, the Examiner states:

> The claims are directed to methods of determining whether a drug or substrate is an inhibitor of fatty acid transporter FATP, using cells transfected with an expression construct encoding a FATP transporter. The specification discloses a FATP protein having an amino acid sequence shown in SEQ ID NO: 25, as well as assays for identifying FATP ligands using cells recombinantly expressing SEQ ID NO: 25. The scope of the patent protection sought by the Applicant as defined by the claims fails to correlate reasonably with the scope of enabling disclosure set forth in the specification...This rejection can be overcome if Applicants amend the claims to recite specific Sequence ID Numbers.

With regard to Claims 104 and 114-116, at pages 6-7 of the Office Action, the Examiner states:

> The specification discloses an enabled utility for the fatty acid transporter encoded by the DNA of SEQ ID NO: 24 and 46, as to be used to identify inhibitors of the transporter. However, there is no discussion, or working examples disclosed in the instant case, as to what amino acids are necessary to maintain the functional characteristics of the claimed polynucleotides encoding the FATP transporter polypeptide. The instant case claims altering as much as 5% of the polynucleotide encoding the polypeptide of SEQ ID NO: 25.

Thus, it appears that both rejections for lack of adequate enablement are based on the Examiner's conclusion that while the teachings in the specification are sufficient to teach the ordinarily skilled artisan how to practice the claimed methods using the polynucleotides with the nucleic acid sequences of SEQ ID NO: 24 and SEQ ID NO: 46, and the polypeptide with the amino acid sequence of SEQ ID NO: 25, the teachings are not sufficient to teach the ordinarily

skilled artisan how to practice the claimed methods using any variant of those sequences. To support this conclusion, the Examiner comments at page 7 of the Office Action that the "art shows that receptor families have members with high structural similarities, but disparate functions", and briefly summarizes the findings of several journal references to show that undue experimentation would be required to practice the invention as claimed.

Although Applicants appreciate the Examiner's indication of enablement for claims directed to particular numbered sequences, Applicants disagree with the Examiner's conclusion regarding the remainder of the claims and specifically traverse the rejection for lack of adequate enablement on the grounds that the specification of the application contains teachings which are more than sufficient to enable the ordinarily skilled artisan to practice the methods throughout the entire scope of the pending claims. The following paragraphs summarize the enabling guidance contained throughout the application and its relevance to the rejections of record. Applicants also discuss how the various references cited by the Examiner actually support a conclusion that the present invention as claimed is fully enabled, rather than the contrary conclusion reached by the Examiner.

This application describes a family of evolutionarily conserved FATPs that mediate the transport of long chain fatty acids (LCFAs) across cell membranes into cells. Throughout the application, teachings are directed to the importance of the structural homology between members of the FATP family and the ability to transport LCFAs. These teachings correlate the structure of the FATPs, *e.g.*, the primary nucleotide or amino acid structure, with the functional characteristics of the FATP, *e.g.*, the ability to transport fatty acids. In numerous studies, Applicants identified additional FATP proteins and polypeptides by comparing the structures of the unknown compounds to the structures of known FATP proteins and polypeptides, and in those studies, the compounds with highly homologous structures functionally performed in a manner which confirmed the FATP family member relationship. In Example 4, sequences conserved among five murine FATP genes were used to search for FATP genes in other organisms. FATP genes were identified in *C. elegans* and *M. tuberculosis*. Both *C. elegans* genes and the *M. tuberculosis* gene were cloned and expressed, and both demonstrated significant LCFA transport capabilities.

Certain of the conserved FATP sequences are described at page 6 of the specification, and illustrated in Figure 1, as an FATP "signature sequence" which is about 360 amino acids in length. As discussed in Example 5, the majority of this FATP signature sequence is unique to FATPs. Absent one short stretch of the FATP signature sequence which contains a relatively common AMP-binding motif, the conserved regions of the FATP signature sequence are not found in any other class of proteins. A segment of this sequences was utilized to construct a phylogenetic tree (see Figure 5), and Figure 6 shows a comparison of the signature sequences of several family members. Likewise, a consensus sequence based on the comparison of 23 independent sequences containing this signature sequence is shown in Figure 90. Thus, this signature sequence is an important criteria, useful in conjunction with overall sequence identity, for placing a protein or polypeptide within the FATP family.

The application also contains numerous working examples demonstrating that FATP family members of various origins and sizes transport fatty acids across cell membranes. Example 2 and Figure 2 describe the results of experiments in which COS cells transfected with murine FATP1, FATP2 and FATP5 demonstrated increased uptake of a fatty acid. Murine FATP1, which has an encoding DNA with a sequence identity of 85 percent when compared to that of human FATP1, was able to effectively transport fatty acids across the membrane of a primate cell. Figure 4 depicts results showing increased fatty acid uptake in *E. coli* cells transfected with tuberculin FATP. Similarly, Figures 37 and 38 also depict results showing increased uptake of fatty acids in cells after transfection with human FATP1 and FATP4.

The specification also contains the results of numerous studies assessing the hydrophilic potential of portions of various FATP proteins (see Figures 92 and 93). This property is particularly relevant to membrane binding potential, and is not only indicative of transport capabilities, but is also useful in identifying potential inhibitors of such transport.

Therefore, it is readily apparent that the claims at issue are fully enabled by the teachings contained in the specification. Such teachings include specific examples of sequences of FATP family members, approximately a hundred in number; complete and partial nucleic acid and protein sequences; illustrative comparisons of the sequence homology between family members and explanations regarding its relationship to tissue expression; and a lengthy signature sequence common to family members. The application also demonstrates that the functions of FATPs are

intimately related to their structure. Therefore, given the extensive teachings in the application, once the skilled artisan has determined that a sequence is a member of the FATP family, the artisan is assured of the "reasonable expectation of success" in practicing the claimed methods which the Federal Circuit has determined is required to meet the enablement standard (see *In re Wright*, 999 F2d 1557, 1564 (FC 1993)). It is respectfully requested that the rejection for lack of enablement be reconsidered and withdrawn.

Furthermore, the references cited by the Examiner, in fact, further support a conclusion of enablement. Smith *et al.* (1997, *Nature Biotechnology 15*:1222-1223) continues his discussion regarding protein families at page 1223 by advising that to avoid errors in correlating structure with function "sequence similarity should be identified via shared conserved sequence patterns or profiles that have been carefully annotated, consistent with the entire family characterized by that pattern".

Brenner (1999, *Trends in Genetics 15*:132-133) was describing attempts to automatically assess gene function solely from database information without any laboratory experimentation at all (see page 132). Such concerns are hardly relevant to the present FATP family which has been extensively studied in the laboratory.

Bisson *et al.* (1993, *Crit Rev Biochem Mol Biol 28*:259-308 is a relatively early study, which acknowledged that much was unknown about glucose transporters in yeast. But, Bisson *et al.* did conclude that while much remained to be learned about the roles and functional importance of specific residues and regions in the yeast transporter proteins, the "value in recognizing the similarity among these proteins is that when a highly conserved residue or region is found to participate in a specific attribute of one protein (e.g., membrane localization or substrate affinity), then it is reasonable to predict that it will play a similar role in the homologous proteins." Thus, Bisson *et al.* supports the conclusion that variants which are FATP family members will transport LCFAs.

Only when Liang *et al.* (1998, Liang, H. *et al. Mol Cell Biol 18:*(2):926) made amino acid substitutions in regions known to be important in glucose transport was substrate specificity affected. Other substitutions made in regions not known to be important in glucose transport had little impact (see page 930). Therefore, Liang *et al.* informs the ordinarily skill artisan that changes which affect the well identified conserved regions of a FATP protein are more likely to

influence FATP transport activity than are changes in other regions. Since the present application clearly describes those regions Liang *et al.* also supports a conclusion of enablement.

Finally, Atsushi Uchiyama, *et al.* (1996, *J Biol Chem 27*:30360-30365) in Figure 8 merely compares a VLACS sequence with a FATP sequence. Applicants were unable to find mention of changing residues to produce a fatty acid transporter. Applicants note that because the VLACS sequence has only about 40% sequence identity with the FATP to which it was compared, and because the two sequences do not appear to share the conserved regions of the FATP signature sequence, Figure 8 does not support a conclusion that a change of less than 3% of the total residues of the VLACS would result in a functional fatty acid transporter.

Therefore, the cited references support a conclusion that when members of a protein family share sequence homology related to a functional characteristic, variants and other family members which share similar sequence homology are also likely to share the related functional characteristics.

Because the application as filed contains teachings and guidance which enable the ordinarily skilled artisan to practice the invention through the full scope of the present claims, and because the cited references also support a finding of enablement, it is respectfully requested that the rejection for lack of enablement be reconsidered and withdrawn.

Furthermore, the amended claims now specify that the FATP is one which has "FATP1 activity". Given the teachings in the specification regarding the close homology between the identified subspecies of the FATPs, such as the FATP1s, and the teachings known in the art regarding the relationships of structure and function, the methods of the present claims are, in fact, fully enabled. Thus, reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,
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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph at page 1, lines 3-8 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

This application is a continuation-in-part of U.S. Patent Application [Number] No. 09/232,201 filed January 14, 1999, now U.S. Patent Number 6,348,321, which claims the benefit of U.S. Provisional Application No. 60/110,941 filed December 4, 1998; U.S. Provisional Application No. 60/093,491 filed July 20, 1998; and U.S. Provisional Application No. 60/071,374 filed January 15, 1998. The teachings of each of these referenced applications are incorporated herein by reference in their entirety.

Please replace the paragraph at page 32, lines 8 through 13 continuing to page 33, lines 1 through 16 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The comparison of sequences and determination of percent identity and similarity between two sequences can be accomplished using a mathematical algorithm. (*Computational Molecular Biology*, Lesk, A.M.,ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects,* Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part 1,* Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology,* von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereaux, J., eds., M. Stockton Press, New York, 1991). In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package [(available at http://www.gcg.com)] (available on the worldwide web at gcg.com), using either a

Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (Devereux, J., *et al.*, *Nucleic Acids Res. 12(1)*:387 (1984)) [(available at http://www.gcg.com)], using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*CABIOS, 4*:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

Please replace the paragraph at page 33, lines17 through 28 continuing to page 34, lines 1 through 2 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (*J. Mol. Biol. 215*:403-10 (1990)). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, word length = 12 to obtain nucleotide sequences homologous to (with calculatably significant similarity to) the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, word length = 3 to obtain amino acid sequences homologous to the proteins of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, *(Nucleic Acids Res. 25*(17):3389-3402 (1997)). When utilizing BLAST and gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. [See http://www.ncbi.nlm.nih.gov.] (see the worldwide web at ncbi.nlm.nih.gov)

Please replace the paragraph at page 34, lines 3 through 11 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Similarity for nucleotide and amino acid sequences can be defined in terms of the parameters set by the Advanced Blast search available from NCBI (the National Center for Biotechnology Information[; see, for Advanced BLAST page,www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast?Jform=1)]. (see, for Advanced BLAST the worldwide web at.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast?Jform=1) These default parameters, recommended for a query molecule of length greater than 85 amino acid residues or nucleotides have been set as follows: gap existence cost, 11, per residue gap cost, 1; lambda ratio, 0.85. Further explanation of version 2.0 of BLAST can be found on related website pages and in Altschul, S.F. *et al., Nucleic Acids Res. 25*:3389-3402 (1997).

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

100. (Amended) A method for identifying an agent which is an inhibitor of fatty acid uptake by a protein, said protein having FATP1 [fatty acid transport] activity and encoded by a polynucleotide which hybridizes to a complement of the polynucleotide of SEQ ID NO: 24 under [stringency] stringent conditions comprising incubation in [of] 6X SSC at 65° C, followed by two or more washes in 0.2X SSC/0.5% SDS at 65° C, comprising the steps of:

a) maintaining test cells expressing said polynucleotide in the presence of a fatty acid and an agent to be tested as an inhibitor of fatty acid uptake;

b) measuring uptake of the fatty acid in the test cells; and

c) comparing uptake of the fatty acid in the test cells with uptake of the fatty acid in suitable control cells;

wherein lower uptake of the fatty acid in the test cells compared to uptake of the fatty acid in the control cells is indicative that the agent is an inhibitor of fatty acid uptake by said protein.

101. (Amended) A method of identifying an agent which is an inhibitor of fatty acid uptake by a protein, said protein having FATP1 [fatty acid transport] activity and encoded by a polynucleotide which hybridizes to a complement of the polynucleotide of SEQ ID NO: 46 under [stringency] stringent conditions comprising incubation in [of] 6X SSC at 65° C, followed by two or more washes in 0.2X SSC/0.5% SDS at 65° C, comprising the steps of:

a) maintaining test cells expressing said polynucleotide in the presence of a fatty acid and an agent to be tested as an inhibitor of fatty acid uptake;

b) measuring uptake of the fatty acid in the test cells; and

c) comparing uptake of the fatty acid in the test cells with uptake of the fatty acid in suitable control cells;

wherein lower uptake of the fatty acid in the test cells compared to uptake of the fatty acid in the control cells is indicative that the agent is an inhibitor of fatty acid uptake by said protein.

104. (Amended) A method for identifying an agent which is an inhibitor of fatty acid uptake by a protein, said protein having FATP1 [fatty acid transport] activity and comprising an amino acid sequence having at least about 95% amino acid sequence identity with the amino acid sequence of SEQ ID NO: 25, comprising the steps of:

a) maintaining test cells expressing said polynucleotide in the presence of a fatty acid and an agent to be tested as an inhibitor of fatty acid uptake;

b) measuring uptake of the fatty acid in the test cells; and

c) comparing uptake of the fatty acid in the test cells with uptake of the fatty acid in suitable control cells;

wherein lower uptake of the fatty acid in the test cells compared to uptake of the fatty acid in the control cells is indicative that the agent is an inhibitor of fatty acid uptake by said protein.

110. (Amended) A method for identifying an agent which is an inhibitor of a protein, said protein having FATP1 [fatty acid transport] activity and being encoded by a polynucleotide comprising a nucleotide sequence which hybridizes to a complement of the polynucleotide of SEQ ID NO: 24 under [stringency] stringent conditions comprising incubation in [of] 6X

SSC at 65° C, followed by two or more washes in 0.2X SSC/0.5% SDS at 65° C, comprising the steps of:

(a) introducing into host cells one or more vectors comprising a polynucleotide expressing said protein;

(b) culturing a first aliquot of the host cells with fatty acid substrate of said protein and with an agent being tested as an inhibitor of said protein;

(c) culturing a second aliquot of the host cells with fatty acid substrate of said protein;

(d) measuring, in the first and second aliquots, uptake of the fatty acid substrate of the host cells;

wherein less uptake of the fatty acid substrate in the first aliquot compared to the second aliquot is indicative that the agent is an inhibitor of said protein.

111. (Amended) A method for identifying an agent which is an inhibitor of a protein, said protein having FATP1 [fatty acid transport] activity and being encoded by a polynucleotide comprising a nucleotide sequence which hybridizes to a complement of the polynucleotide of SEQ ID NO: 46 under [stringency] stringent conditions comprising incubation in [of] 6X SSC at 65° C, followed by two or more washes in 0.2X SSC/0.5% SDS at 65° C, comprising the steps of:

(a) introducing into host cells one or more vectors comprising a polynucleotide expressing said protein;

(b) culturing a first aliquot of the host cells with fatty acid substrate of said protein and with an agent being tested as an inhibitor of said protein;

(c) culturing a second aliquot of the host cells with fatty acid substrate of said protein;

(d) measuring, in the first and second aliquots, uptake of the fatty acid substrate of the host cells;

wherein less uptake of the fatty acid substrate in the first aliquot compared to the second aliquot is indicative that the agent is an inhibitor of said protein.

114. (Amended) A method for identifying an agent which is an inhibitor of a protein, said protein having FATP1 [fatty acid transport] activity and comprising an amino acid sequence having

at least about 95% amino acid sequence identity with the amino acid sequence of SEQ ID NO: 25, comprising the steps of:

(a) introducing into host cells one or more vectors comprising a polynucleotide expressing said protein;

(b) culturing a first aliquot of the host cells with fatty acid substrate of said protein and with an agent being tested as an inhibitor of said protein;

(c) culturing a second aliquot of the host cells with fatty acid substrate of said protein;

(d) measuring, in the first and second aliquots, uptake of the fatty acid substrate of the host cells;

wherein less uptake of the fatty acid substrate in the first aliquot compared to the second aliquot is indicative that the agent is an inhibitor of said protein.